BEACON ANALYTICAL SYSTEMS
AFLATOXIN PLATE KIT

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GENERAL INFORMATION

Aflatoxin Plate Kit is a competitive ELISA for the quantitative analysis of Aflatoxin (5 – 100 ppb) in corn.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	Beacon Analytical Systems Inc. 207-571-4302
Test Kit Name:	Aflatoxin Plate Kit
Product Number:	20-0017
Effective Date of Instructions:	5/14/2015
Instructions Revision Number	0
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn
Extraction Method:	Blend 50 gram sample with 5 gram sodium chloride (NaCl) and 100 milliliters (mL) of 80% Methanol/20% distilled or deionized water (v/v) for 1 minute.
Test Format:	Microtiter well plate assay
Detection Method:	Stat Fax Reader, Model 303 Plus

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Reagents: Allow kit reagents (Aflatoxin-HRP Conjugate, Calibrators, Antibody, Substrate, Stop Solution and plate) to reach room temperature prior to running the test.

b. Preparation of Extraction Solvent [80% Methanol/20% Water (v/v)]

The extraction solvent used in the method is a methanol/water mixture consisting of 80% methanol (reagent grade or better) and 20% distilled or deionized water (v/v).

1. Using a 1000 mL graduated cylinder, measure 800 mL methanol and place it into a clean carboy with spigot.
2. Using a 250 mL graduated cylinder, measure 200 mL distilled or deionized water and add to the methanol. Shake until it is completely mixed.
3. Label the container stating the mixture 80% methanol/20% water (v/v), date of preparation, and initials of technician who prepared the solvent.
4. Store this solvent at room temperature in a tightly closed container until needed. Mix again before use.

   NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts methanol to 2 parts distilled or deionized water.

Stat Fax 303 Plus reader calibration:

1. Turn the power switch on and look for **Ready** on the screen.
2. Press **ABS** and Press **2** (450 nm) and **Enter**.
3. Press **4** (630 nm) and **Enter** and then place the empty carrier on the track with alignment.
4. Press **Enter** and see the values (Max ± 0.005).
5. Press **Clear** twice to quit, perform this procedure every time you turn the reader on.

Set up for an initial test (this is one time procedure):

1. Press **ALT** and then press **YES** when STRIP TYPE Y/N displayed.
2. Press **YES** when 8 wells Y/N displayed.
3. The display shows Ready then Press **MULT**.
4. Press **YES** when Regression Y/N displayed.
5. The display shows SELECT FILTER and press **2** for 450 nm and **Enter**.
6. The display shows SELECT DIF FILTER and press **4** for 630 nm and **Enter**.

7. Press **NO** when Linear Y/N displayed.
8. Keep selecting **NO** until Log/Logit Y/N displayed.
9. Press **YES** when Log/Logit Y/N displayed.
10. Press **NO** when BLANK Y/N displayed.
11. Press **NO** when Duplicates Y/N displayed.
12. Press **5** and **Enter** when # of Cals displayed.
13. Press **YES** when Select Units Y/N displayed.
14. Press **12** and **Enter** when Key unit code # displayed.
15. Press **YES** when ppb Y/N displayed.
16. Press **2.0** and **Enter** when Cal2 displayed.
17. Press **7.5** and **Enter** when Cal3 displayed.
18. Press **25** and **Enter** when Cal4 displayed.
19. Press **100** and **Enter** when Cal5 displayed.
20. Press **NO** when Off Curve Ok Y/N displayed.
21. The display shows Set Carrier to 1.
22. Put a strip with Calibrators (and samples) in the carrier (right position). Press **Enter**.
    (0 ppb of Calibrator is read first)
23. Wait until lamp warmed up.
24. Press **NO** when PLOT Curve Y/N displayed.
25. Press **YES** when Accept Curve Y/N displayed.
    (Only if the R^2 value is higher than 0.990)
26. The display shows Set Carrier.
27. Press **ALT**.
28. Press **YES** when SAVE TEST Y/N displayed.
29. Press **YES** when NAME TEST Y/N displayed.
30. Select character by pressing 4 or 6. Press **Enter**.

31. Repeat this step to complete the TEST name.
    (Press **Enter** a second time immediately after the last character was chosen)

32. The TEST will be saved as TEST # 1 unless there are any other tests saved previously.

EXTRACTION PROCEDURES

1. Weigh 50 ± 0.2 grams ground sample in a blender jar.
2. Add 5 grams sodium chloride (NaCl).
3. Add 100 mL of extraction solvent.
4. Blend for 1 minute at high speed.
5. Filter through a coffee filter.
6. Dilute 5 mL of filtrate with 20 mL of distilled or deionized water and mix thoroughly.
7. Filter through a glass fiber filter (Fisher Scientific G6 or equivalent).
8. Collect the second filtrate for testing.
8. Run the assay within 1 hour in order to keep the integrity of the extract.
9. Proceed to TEST PROCEDURES.

TEST PROCEDURES

1. Place the appropriate number of test wells into the microwell holder for all Calibrators and samples to be tested.
2. Mix each reagent by swirling the reagent bottle prior to use.

Example

Well Location	1	2	3	4	5	6	7	8
Calibrators and Samples	C 0 ppb	C 2.0 ppb	C 7.5 ppb	C 25 ppb	C 100 ppb	Test Sample	Test Sample	Test Sample

3. Add 50 µL of Aflatoxin-HRP Conjugate to each test well.

4. Using a new pipette tip for each, transfer 50 µL of each calibrator (0, 2.0, 7.5, 25, and 100 ppb) and samples to the designated wells.

5. Using a multi-channel pipettor, transfer 50 µL of antibody to each wells.

6. Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing the reagents from the wells. Incubate for 10 minutes at room temperature

7. Dump contents, and wash with distilled or deionized water 5 times.

8. Invert and tap on the paper towel to remove all remaining water.

9. Pour the needed volume of substrate into the reagent boat.

10. With new tips on the multi-channel pipettor, prime and pipette 100 µL of substrate into the wells and mix by sliding back and forth on a flat surface for 10-15 seconds. Incubate for 10 minutes.

11. With new tips on the multi-channel pipettor, prime and pipette 100 µL of stop solution into the wells and mix by sliding back and forth on a flat surface for 10-15 seconds.

12. Wipe the bottom of the microwells with a lint free dry Kim wipe and read on the Stat Fax reader using a 450 nm. Air bubbles should be eliminated, as they could affect analytical results.

Reading the test results:

1. Press **Menu**.

2. Press **1** and **Enter** when Select Test displayed.

3. Press **NO** when Plot Curve Y/N displayed.

4. Press **NO** when Stored Curve displayed.

5. The display shows Set Carrier to 1.

6. Put a strip with Calibrators and samples in the carrier (right position).
(0 ppb of Calibrator is read first)

7. Press **Enter**.

8. Press **NO** when Plot Curve Y/N displayed.

9. Press **YES** when Accept Y/N displayed.
(Only if the R^2 value is higher than 0.990)

10. Concentrations of the samples will be calculated and printed on the paper.

11. Put a second strip with samples to read in the middle line.
    (Move the carrier to right)

12. Press **Enter**.

13. Concentrations of the samples will be calculated and printed on the paper.

14. If there are more samples to read, repeat the procedures (step 11 and 12) with a new strip or Press **Clear** twice to quit.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (corn only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation. The range for performance evaluation of quantitative aflatoxin test kits is 5 – 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 5 – 100 ppb for aflatoxins), and a correction for dilution is applied to derive at the final result. For this test kit, the appropriate calibration setting is selected for automatic correction for the supplemental dilution performed. Supplemental analysis is performed only at the request of the applicant.

Supplemental analysis procedure:

1. Prepare 16% Methanol solution by mixing 1 part of the extraction solution with 4 parts of distilled or deionized water. Mix well and store tightly sealed container.

2. Dilute the final extract after the second filtration (step 8 in EXTRACTION PROCDURES) with this 16% Methanol as desired. Run the assay with this diluted extract.

3. Apply the dilution factor to calculate the concentration of Aflatoxin in the sample.

4. If you mixed **1** part of extract with **1** part of 16% Methanol. This is a 1 to 2 dilution (v:v), and the dilution factor is 2. Multiply the result by dilution factor of **2** to obtain actual Aflatoxin concentration in the sample.

A final result less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

EQUIPMENT AND SUPPLIES

1. Material provided in the Beacon Aflatoxin Plate test kit.

The test kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8º C (1 year from the date of manufacture).

a. 1 Plate containing 12 test strips of 8 wells each (total 96 wells), vacuum- packed in aluminized pouch with indicating desiccant.

b. 5 vials, each containing 2 mL of Aflatoxin Calibrators corresponding to 0, 2.0, 7.5, 25 and 100 µg/L (ppb) Aflatoxin.

c. 1 vial containing 8 mL of Aflatoxin-HRP Enzyme Conjugate.

d. 1 vial containing 8 mL of Aflatoxin Antibody Solution.

e. 1 vial containing 14 mL of Substrate.

f. 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

g. 1 Instructional Booklet.

2. Materials Required but not Provided.

a. Distilled or deionized water.

b. Methanol - ACS grade or better.

c. Graduated cylinder, 100 mL and lager.

d. Coffee filter.

e. Fisher Scientific G6 (#09-804-110A) or equivalent glass fiber filter.

f. Warring high-speed blender with a jar, or equivalent.

g. Stat-Fax Reader Model 303 Plus equipped with a 450-nm filter.

h. Multi –channel pipette, (8 channels) with pipette tips capable of dispensing 50 µL, and 100 µL.

i. Serological pipette (10 mL) or any pipette capable of dispensing 5 mL.

j. Vortex mixer.

k. Wash bottle and Timer.

STORAGE CONDITIONS AND PRECAUTIONS

1. **Storage Conditions**

   a. The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 2 -8° C. **(DO NOT FREEZE)**

   b. Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.

   c. The Substrate / chromogen solution is light sensitive, therefore, avoid exposure to direct light.

2. Precautions

   a. Each reagent is optimized for use in the Beacon Aflatoxin Plate Kit. Do not combine reagents from other Beacon Aflatoxin Plate Kits with different Lot numbers.

   b. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.

   c. Do not use reagents after expiration date.

   d. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.

   e. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. If contact should occur, immediately flush with copious amounts of water. Immediately clean up any spills and wash area with copious amounts of water.

   f. The use of a multichannel pipette to dispense the generic reagents (Conjugate, Antibody, Substrate and Stop Solution) is recommended when running 2 strips or more.

   g. The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon Analytical Systems Inc. (www.beaconkits.com) for technical support if you have any questions about the use of this kit.

REVISION HISTORY

Revision 0 (5/14/2015)